Fish Physiol Biochem (2006) 32:249–253
DOI 10.1007/s10695-006-9101-x

ORIGINAL PAPER

Embryonic expression of UCP2 in rainbow trout (*Oncorhynchus mykiss*)

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Received: 10 February 2006 / Accepted: 30 July 2006 / Published online: 13 September 2006

Abstract Uncoupling proteins are mitochondrial anions transporters that dissociate respiration from ATP synthesis through proton leaks. Uncoupling protein 2 reportedly plays a role in several physiological processes such as energy partitioning, nutrition, and fatty acid metabolism. The mRNA expression of rainbow trout *UCP2* genes (*UCP2A* and *UCP2B*) was monitored during embryogenesis and early larval development. Both genes were recruited early and displayed similar steadily decreasing patterns from fertilization until hatching. The expression of *UCP2A* and *UCP2B* appeared significantly differentiated after hatching and during the yolk sac absorption, with *UCP2A* displaying higher expression. We suggest that *UCP2* expression profiles in the rainbow trout embryo could be associated with the utilization of lipids as a source of energy during development.

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Keywords Development · Embryo · Fat · mRNA · Trout · UCP2 · Uncoupling protein · Yolk sac

Introduction

Uncoupling proteins (UCPs) are capable of dissipating the proton gradient across the inner mitochondrial membrane to generate heat while reducing the efficiency of ATP synthesis (Ledesma et al. 2002). UCP2 has been described to play a role in various physiological processes such as energy partitioning (Surwit et al. 1998; Fleury et al. 1997), body weight control (Trayhurn 2005; Jaberi 2004; Hagen and Vidal-Puig 2002; Schonfeld-Warden and Warden 2001), fatty acid metabolism (Goglia and Skulachev 2003; Jezek 1999), and control of reactive oxygen species (Echtay et al. 2002; Negre-Salvayre et al. 1997). Two *UCP2* genes were previously characterized in the rainbow trout genome (Coulibaly et al. 2006). The expression of both genes showed a wide range of tissue distribution and appeared differentially regulated in response to fasting in the muscle of juvenile fish. Knowing the reported role of UCP2 in nutrition and energy partitioning, the present study was designed to analyze the changes in *UCP2* mRNA expression during rainbow trout embryonic and larval developments in an attempt to understand the potential role of

the gene in development. Quantitative trait loci for the embryonic development rate have been described to be genetically correlated with the length of fry (Robison et al. 2001; Beacham 1988) and are also associated with large size and early sexual maturation (Sundin et al. 2005; Allendorf et al. 1983). Thus, characterization of candidate genes such as UCP2s that are involved in embryonic development processes could be valuable in discovering their potential for impacting important economic traits in adult fish.

Materials and methods

RNA isolation

Eggs and sperm from 5 female and 2 male rainbow trout were obtained from Trout Lodge (Sumner, WA, USA). Eggs were artificially fertilized and incubated at 13°C in a flow-through system using a photoperiod of 12 h light/12 h dark. Unfertilized eggs and embryos were collected every day for 14 days following fertilization and immediately frozen in liquid nitrogen and stored at – 80°C until RNA extraction. Similar collections were repeated every other day thereafter until hatching, which occurred at 26 days post-fertilization (dpf). After hatching, samples were collected as individual alevins every third day until swim-up of young fry (49 dpf). Samples were powdered using a liquid nitrogen cooled Bessman tissue Pulverizer (Spectruim Laboratories, Rancho Dominguez, CA, USA) and the powder was added to 1 ml of Tri-reagent for RNA extraction. Total RNA was extracted using Tri-reagent (Molecular Research Center, Cincinnati, OH, USA) modified with high salt solution according to the manufacturer's protocol. RNA was treated with DNAse to eliminate genomic DNA contamination and re-extracted using Tri-reagent. RNA concentrations were estimated by spectrophotometry and integrity was observed on agarose gels. First-strand cDNA synthesis was performed in a 40-μl reaction volume containing 2 μg total RNA, 1 μg of random hexamer primers, 1 × M-MLV reaction buffer, 500 μM of dNTPs, 200 units of M-MLV reverse transcriptase (Promega, Madison, WI, USA) and 25 units of Recombinant RNasin Ribonuclease Inhibitor (Promega). The mix was incubated at 37°C for 60 min with a final denaturation at 95°C for 5 min. Reactions were stored at –20°C until further use.

Real-time RT-PCR

Specific forward and reverse primers were designed for *UCP2A* (GenBank DQ295326) and *UCP2B* (GenBank DQ295328) and a reference gene, EF1-α (Table 1). Real time RT-PCR was performed in a 15-μl reaction volume containing 1 μl of cDNA template, 250 nM each of the forward and reverse primers and 7.5 μl of SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA). Amplifications were performed on the ABI PRISM 7900 Sequence Detection System real-time cycler (Applied Biosystems). Non-template controls were included in each run and checked for genomic contamination. The amount of transcript in each sample was quantified using the standard curve method (Rutledge and Cote 2003). Target gene expression in embryos was normalized to *EF1-α* (GenBank Accession Number AF498320.1).

Results and discussion

The mRNA expression of the *UCP2A* and *UCP2B* genes was detected in all samples from unfertilized eggs to 49-day-old alevins. The expression level of our chosen internal reference gene (*EF1-α*) appeared very low in unfertilized eggs and in 1-, 2-, 3-, and 4-day-old embryos before it increased and leveled off at 5 dpf. We

Table 1 *UCP2A*, *UCP2B* and *EF1-α* specific primer sequences for real-time quantitative PCR

Gene	Forward primer 5′ → 3′	Reverse primer 5′ → 3′
UCP2A	TCCGGCTACAGATCCAGG	CTCTCCACAGACCACGCA
UCP2B	TGTAATCACGAGGCATCA	GGATTCTCTAAAGGCGTC
EF1-α	GGGCAAGGGCTCTTTCAAGT	CGCAATCAGCCTGAGAGGT

assessed *18S*, *TBP-α* and *β-actin* mRNAs as alternative internal reference controls for this study and chose *EF1-α* because it showed the most stable real-time PCR Ct (threshold cycle). The mRNA expression patterns of these genes were similar to that of *EF1-α*: initially very low, but increasing and leveling off at day 5. This low expression in the early embryo is consistent with the beginning of zygotic transcription at the midblastula transition step (Kane and Kimmel 1993), which has been described to occur at approximately 3 dpf in rainbow trout (Takeuchi et al. 1999). As a consequence of the low mRNA expression of housekeeping genes, *UCP2A* and *UCP2B* gene expression in unfertilized eggs, and 1-, 2-, 3-, and 4-day-old embryos was not included in Fig. 1, which shows expression starting from 5 dpf. Nevertheless, our results suggest that *UCP2* gene expression precedes midblastula transition in rainbow trout. This could be a result of the maternal *UCP2* gene expression. Maternal factors have been shown to control development before the activation of the embryonic genome in Zebrafish (Dosch et al. 2004). However, data in *Xenopus* showed that zygotic transcription could start prior to the midblastula transition (Kimelman and Kirschner 1987; Yang et al. 2002).

The highest expression of both *UCP2* genes was detected at 5 dpf with the amount of *UCP2A* transcript appearing lower than that of *UCP2B*. Similar early expression of the murine *UCP2* gene has been reported in the fetus. *UCP2* mRNA is reportedly expressed in various tissues of the mouse fetus and was observed to be 30-fold higher in fetal liver than in adult liver (Villarroya et al. 2001; Carmona et al. 1998; Hodny et al. 1998). The relative expression of both *UCP2A* and *UCP2B* genes steadily decreased and at the eyed-egg stage (12 dpf), mRNA expression was 33% and 28% of the values observed at 5 dpf for *UCP2A* and *UCP2B*, respectively. Both genes showed similar relative mRNA expression from 13 dpf to 18 dpf. The amount of *UCP2A* gene transcripts gradually increased 6 days before hatching, while that of *UCP2B* slightly decreased. This trend persisted in the young alevins after hatching. During the whole yolk sac absorption stage (26–39 dpf), although declining, the mRNA expression level of the

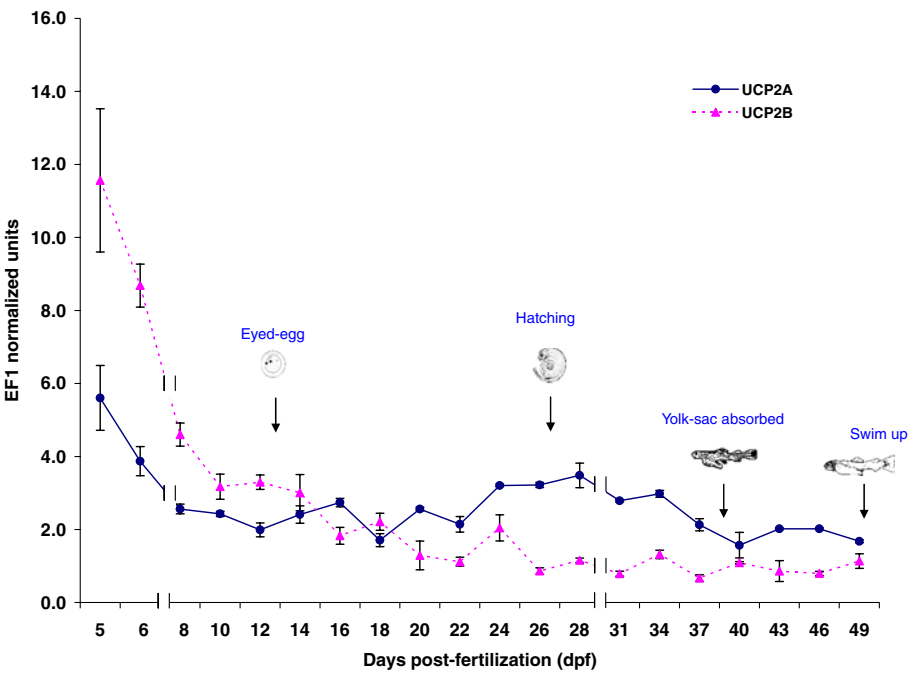

Fig. 1 Relative mRNA expression of *UCP2A* and *UCP2B* estimated by real-time RT-PCR during rainbow trout embryo and larval developments at 13°C. *Arrows* represent developmental stages. Data are represented as mean ± standard error (n = 3–5). Gene mRNA values were normalized to *EF1-α*

UCP2A gene remained much higher than that of the *UCP2B* gene. After complete absorption of the yolk sac, the amount of *UCP2A* gene transcript is lower and still on average twice that of *UCP2B* until first swim-up of the young fries. Several studies have demonstrated the role of UCP2 in fatty acid metabolism (Goglia and Skulachev 2003; Jezek 1999). In the adult rat a high-fat diet induces up-regulation of the *UCP2* gene, thus promoting fat utilization (Samec et al. 1999; Matsuda et al. 1997). The early and intensive recruitment of *UCP2* genes in the rainbow trout embryo could be associated with the use of fat as a source of energy, as developing embryos of fish use their lipid reserves to fulfill their energy requirements (Boulekbache 1981; Haliloglu et al. 2003). *UCP2A* is likely to be more involved than *UCP2B* in the utilization of yolk sac nutrients as a source of energy in young alevins, while the reverse situation was observed before the eyed-egg stage. Overall, it could be suggested that a virtual linear relation exists between the decreasing expression of *UCP2* genes and the decreasing amount of saturated and monounsaturated fatty acids used as energy substrates in rainbow trout developing embryo, as described by Haliloglu et al. (2003).

In conclusion, *UCP2A* and *UCP2B* genes were highly expressed early during embryonic development. The mRNA expression of *UCP2A* and *UCP2B* genes showed similar patterns from fertilization to hatching and appeared differentiated in the following stages. We hypothesize that the expression of both *UCP2A* and *UCP2B* genes in rainbow trout developing embryo and alevin might be associated with the utilization of fatty acids as a source of energy for growth and development.

Acknowledgements We wish to thank Kristy Anderson, Roseanna Athey, and Renee Fincham for their excellent technical assistance. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

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